THE KINETICS OF GSNO DEGRADATION IN RAW 264.7 CELLS

Michelle M. Boockmeier, Yanhong Zhang, Dr. Neil Hogg*
Medical College of Wisconsin
Biophysics Research Institute
8701 Watertown Plank Road,
Milwaukee, Wisconsin 53226
michelle.boockmeier@snc.edu
Biochemistry

ABSTRACT: S-nitrosothiols are compounds that have the generic structure R-SNO. S-nitrosothiols, or SNO's, are most often formed from the nitrosation of thiol species in cells. S-nitrosoglutathione (GSNO) is an S-nitrosothiol formed from the nitrosation of glutathione (GSH). It is possible that the formation of GSNO in cells is a protective mechanism that serves to protect the cell from damage by free-radical species. NADH-dependent GSNO Terminase activity has recently been observed in several organisms. Sequencing data indicates that the enzyme responsible for GSNO degradation is Class III Alcohol Dehydrogenase (ADH III), which is also known as Glutathione-Dependent Formaldehyde Dehydrogenase (FDH). Recently, the degradation of GSNO in cell lysate of RAW 264.7 cells has been observed, prompting a study of the kinetic mechanism of GSNO decay in RAW 264.7 cell lysate. The kinetics of GSNO decay were followed by both UV-VIS spectroscopy and chemiluminescence, and the results were fit to the Michaelis-Menten model of enzyme kinetics. GSNO Terminase activity was found to be localized within the protein fraction of the cell lysate. The kinetics of GSNO decay in RAW 264.7 cell lysate display different characteristics than those of GSNO decay in pure ADH III isolated from RAW 264.7 cells. The kinetic differences indicate that (1) the activity of GSNO Terminase decreases with time, (2) ADH III/FDH is not completely responsible for GSNO decay in cells, and (3) GSNO levels in cells are not sustainable due to high activity of GSNO Terminase.